Control of the Seasonal Arrest of Copulation and Spawning in the Apple Snail *Pomacea canaliculata* (Prosobranchia: Ampullariidae): Differential Effects of Food Availability, Water Temperature, and Day Length

EDUARDO A. ALBRECHT, EDUARDO KOCH, NORMA B. CARREÑO, AND
ALFREDO CASTRO-VAZQUEZ

Laboratory of Physiology (IHEM-CONICET), Department of Morphology and Physiology,
National University of Cuyo, Mendoza, Argentina

Abstract.   Two laboratory experiments were carried out on summer (active) pairs of *Pomacea canaliculata* (Lamarck, 1822), to study the influence of food availability (*ad libitum* feeding and restriction to 25% of the *ad libitum* requirements), and to determine the effects of water temperature (25°C and 18°C) and day length (14 hr light/day and 10 hr light/day) on the frequencies of copulation and spawning, and on some spawn measurements such as egg number and volume, and percentage of fertile eggs (i.e., those containing developing embryos). In all groups, the animals were first observed during 14 days in "acclimation" conditions (i.e., *ad libitum* feeding, 25°C, and 14 hr light/day), and then changed to one of the different "treatments." Both food restriction and low water temperatures induced a decrease in the frequencies of both copulation and spawning, without altering the spawn measurements. Short day length was without effect on these behaviors. We conclude that low food availability and cool water are important cues for the seasonal arrest of copulation and spawning in *P. canaliculata*. We conclude that both food restriction and low water temperatures are responsible for the seasonal arrest of copulatory and spawning behaviors in *Pomacea canaliculata*, although autumn decreasing temperature seems to be the critical factor in the field. Shortening of the photoperiod may be of little or no importance in determining the seasonal reproductive arrest.

INTRODUCTION

The ampullariids are a family of limnic gastropods widely distributed in tropical, subtropical, and temperate zones over the Old and New World (Hylton Scott, 1957; Berthold, 1989). The combined occurrence of a gill and a lung, and their ability to embed themselves in the mud of aquatic environments, permit apple snails to tolerate periods of drought and low water temperatures, and have allowed them to colonize a wide spectrum of waters (Hylton Scott, 1957; Pain, 1972; Castellanos & Fernandez, 1976; Berthold, 1989). *Pomacea canaliculata* (Lamarck, 1822) occurs mainly in lentic habitats throughout the lower Amazon basin and the Plata basin (Hylton Scott, 1957; Martin et al., 2001). It also occupies semi-arid regions in the west of Argentina up to 500 m above sea level, with the 38° parallel as its southern boundary (Cazzaniga, 1987).

This Neotropical species has been introduced (ca. 1980) to several Asian countries for aquaculture, and has settled in rice farms, where it has become a pest (Halwart, 1994; Cowie, 1998). It may also act as an intermediate host of the nematode *Angiostrongylus cantonensis* (Chen) (Leon-Dancel, 1970). The threats posed by this anthropogenic spread of *Pomacea canaliculata* and, on the other hand, the potential use of ampullariid snails in the control of the intermediate hosts of *Schistosoma mansoni* (Sambon) (Cazzaniga, 1990b; Pointier & Giboda, 1999) or as a biological control for aquatic weeds (Thomas, 1975; Cazzaniga, 1983) have raised an increasing interest in its biology.

In the tropical and subtropical zones of Brazil and Paraguay, *Pomacea* species seem to be active throughout the year (Hylton Scott, 1957; Fausto Filho, 1962, 1965; Milward de Andrade et al., 1978) while in field populations of *Pomacea canaliculata* in southern South America, copulation and spawning occurs only during the warm season, and breeding ceases in autumn (Estebenet & Martín, 2002). Albrecht et al. (1999) showed that increases in water temperature induce the onset of both copulation and spawning activities in winter inactive individuals of *Pomacea canaliculata*, while a shift to a long photoperiod was ineffective to do so; also, food restriction did not significantly affect the activation induced by temperature rise. Estebenet & Cazzaniga (1992, 1993) reported that winter conditions delayed growth and sexual maturation in this species, and they also observed that spawning frequency was also decreased. However, they did not determine whether such effects were due to changes either in water temperature, shifting to a short photoperiod, or a decrease in food intake.

To approach the question of which environmental factors are responsible for the seasonal arrest of reproduction

we investigated the effects of food availability, water temperature, and day length on copulation and spawning in summer active pairs of *Pomacea canaliculata* under laboratory conditions.

MATERIALS AND METHODS

Maintenance of Snails in the Laboratory

Sexually mature individuals of *Pomacea canaliculata*, of both sexes, were collected in Palermo Park (Buenos Aires, Argentina) during the spring/summer of 1993–1996. Voucher specimens of this population were deposited at the collection of the Museo Argentino de Ciencias Naturales (Buenos Aires, Argentina; lot MACN-In 35707). The snails were maintained in outdoor ponds until they were used for studies. The observations were conducted in four aquaria (56 × 41 × 20 cm), each divided into 10 compartments (11 × 20 × 20 cm) with a plastic mesh. The shell lengths ranged from 33–67 mm (sexual maturity is reached at about 25 mm, Martín, 1986; Estebenet & Cazzaniga, 1992). One male and one female (sex was determined by the shape of the operculum, Cazzaniga, 1990a, and confirmed by autopsy after the experiments) were placed in each compartment. Continuous water renewal about 10 times/day was ensured by a constant input flow, and water level was kept 10 cm above the bottom. The aquaria were illuminated with 15 W white fluorescent tubes located 22 cm above the water. Snails that died during the observations were replaced by another individual of the same sex, since previous observations (Albrecht & Castro-Vazquez, unpublished) indicated that neither the frequency of copulation nor that of spawning change after replacement of one partner.

Acclimation Period

Prior to each experiment the pairs were allowed to acclimate to the aquaria for 2 weeks during which the snails were maintained on a photoperiod of 14 hr of light/day, which is similar to the longest day of the year in Mendoza. Water temperature was kept at 25.5 ± 1.9°C (mean ± SD), which approximates maximum water temperatures at noon during summer (based on 1992–1993 recordings in our outdoor ponds) and fresh lettuce was provided *ad libitum*.

Observations of Snails

Daily observations of the pairs were made at 7–9:30 a. m.; 1:30–2:30 p. m.; and 6:30–9 p. m. (the middle of the illuminated period was fixed at noon) during both the acclimation and the experimental period of 14 days during which copulation and spawning were recorded. Only males that copulated at least twice (92/115, 80%), and females that spawned at least once (92/115, 80%) during the acclimation period were considered as active individ-

uals and were included in the study. The snails were alloted at random to the different experimental groups.

A single episode of copulation was recorded if copulatory activity was observed on either one or more than one consecutive observations on the same pair. An episode of spawning was recorded when a female was observed during egg-laying or if an egg mass had been deposited between consecutive observations (*P. canaliculata* females lay their eggs above the water level). Frequencies were calculated as the slope of the cumulative curve of episodes of either copulation or spawning. Differences between means were evaluated with a one-tailed Mann-Whitney U-Test (when two groups were compared) or Kruskal-Wallis Analysis of Variance by Rank, followed by the Mann-Whitney U-Test for subsequent individual comparisons (Siegel & Castellan, 1988). Significance level was fixed at $P < 0.05$.

Only results for those snails that remained alive during the whole acclimation and experimental periods are presented.

Measurements of Spawns

The spawns were collected as soon as they were observed. The egg masses were dispersed in 2% sodium hydroxide, washed, and air-dried thoroughly before counting the eggs (Albrecht et al., 1996). The mean egg volume was calculated on the basis of the mean diameter of a 10 egg-sample from each spawn, measured with a caliper to the nearest 0.1 mm. The spawn volume was calculated as the product of the mean egg volume and the number of eggs in the spawn. The eggs were incubated in an air-conditioned room at 24 ± 2°C for 10–15 days, when the proportion of developing embryos was determined for each spawn. The differences in spawn measurements between treatments were evaluated with either the Student's t Test for independent samples (when two groups were compared) or with ANOVA I, followed by the Tukey Test (Zar, 1984). Angular (arcsine %x) transformation was used for percentages of developing embryos(x).

Effect of Food Availability on Copulation and Spawning

After the acclimation period, two experimental treatments were applied during the following 14 days: (1) Control—the snails were fed *ad libitum* with fresh lettuce. The amount of fresh lettuce eaten was experimentally determined as 170 g/kg live mass, and was used as a reference for the restricted group; (2) Restricted—snails were fed with 42.5 g/kg live mass, i.e., 25% of the amount the control snails received.

One female that was exposed to restricted feeding (1/14, 7%) died during the experimental period. No male died during the same period.
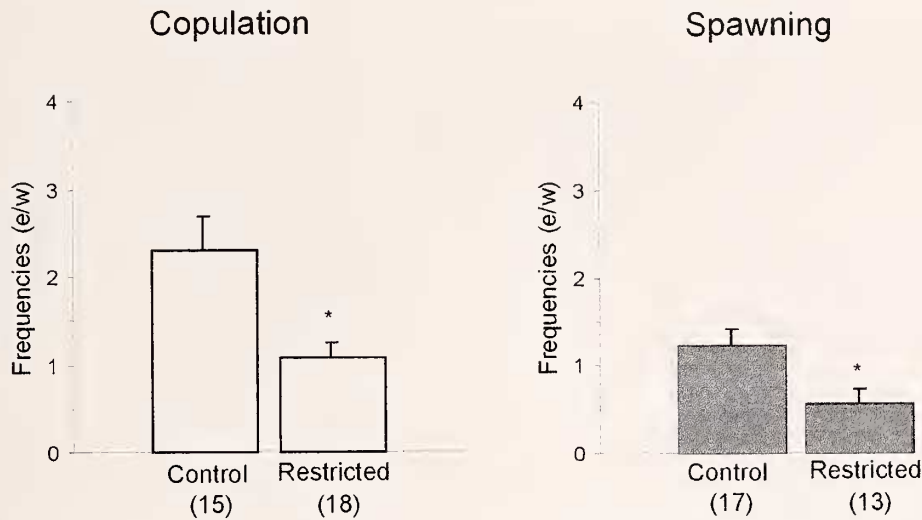
Copulation

Spawning

Figure 1.   Effect of food availability on the mean frequencies (± SE) of copulation and spawning (episodes/week by mating pair). All animals were maintained in warm water and under a long photoperiod. Number of cases is indicated between parentheses. Asterisks indicate (in the same panel) significant differences ($P < 0.05$, Mann-Whitney U Test).

Effects of Water Temperature and Day Length on Copulation and Spawning

The low water temperatures were chosen within the range observed during April (early autumn) in our outdoor ponds (10–20°C; 1992–1993 recordings). Day lengths were chosen to approximate the longest and the shortest day of the year in Mendoza.

Four experimental treatments were set up as follows: (1) WL, warm water (25.5 ± 1.9°C, mean ± SD) and long photoperiod (14 hr light/10 hr dark); (2) WS, warm water and short photoperiod (10 hr light/14 hr dark); (3) CL, cold water (18.5 ± 1.9°C, mean ± SD) and long photoperiod; and (4) CS, cold water and short photoperiod. The snails were fed with fresh lettuce *ad libitum* throughout the experiment.

Four males died on day 5 of the experimental period. All of them had been exposed to the lower water temperature (18°C); three of them had pertained to the short photoperiod group (3/18, 17%), while one had been exposed to the long photoperiod (1/16, 6%).

Replicate observations were run for both experiments, starting on February 8, 1994; March 31, 1994; January 22, 1995; March 31, 1995; and February 26, 1996. Since no differences were apparent between the sets of observations, they were pooled for presentation.

RESULTS

Effect of Food Availability on Copulation and Spawning

The frequencies of both copulation and spawning were significantly lower when the animals were fed on a restricted amount of food (copulation: Mann-Whitney U Test, $U = 73.00$, P = 0.024; spawning: Mann-Whitney U Test, $U = 58.00$, P = 0.028; Figure 1). No significant differences between treatments were found (Student's t Test for independent samples, $P > 0.05$) in either the number of eggs per spawn, the mean egg volume, the percentage of developing embryos, or the volume of the spawn (Table 1).

Effects of Water Temperature and Day Length on Copulation and Spawning

The frequencies of both copulation and spawning significantly decreased (copulation: Kruskal-Wallis Test: H (df = 3, n = 59) = 22.43, $P = 0.0001$; spawning: Kruskal-Wallis Test: H (df = 3, n = 62) = 22.24, $P = 0.0001$; Mann-Whitney U Test for subsequent individual comparisons, $P < 0.05$; Figure 2) after the exposure to the low water temperature under both, long and short photoperiod.

No significant differences were found (ANOVA I, Tukey Test; $P > 0.05$) in any of the spawn measurements (number and volume of eggs, volume of the spawn, and percentage of developing embryos) between the different experimental groups (Table 2).

DISCUSSION

Water temperature, day length, and availability of food were investigated as likely candidates for determining the seasonal arrest of copulation and spawning in *Pomacea canaliculata*. Water temperature may be a better environmental cue than air temperature to predict the coming winter, since water bodies cool off slowly and steadily in autumn in contrast to the greater fluctuations of air temperature (Daly & Wilson, 1983). Day length is also a

Table 1

Spawn measurements in females fed with either a control (*ad libitum*) or a restricted diet. All animals were maintained in warm water and under a long photoperiod. Results are expressed as the "weighted" mean (± SE) and the range of the mean values of individual females.

	ad libitum feeding (13 females, 42 spawns)		Restricted feeding (7 females, 15 spawns)	
	Mean ± SE	Range	Mean ± SE	Range
Number of eggs per spawn	115.1 ± 14.7	28.0–229.7	125.6 ± 12.2	70.0–185.5
Mean egg volume (mm³)	10.1 ± 0.7	6.7–15.9	10.1 ± 0.4	7.6–11.7
Spawn volume (mm³)	1134.1 ± 145.5	318.4–2389.2	1292.2 ± 148.6	735.4–2004.1
% of developing embryos	35.1 ± 4.9	0.0–59.1	41.6 ± 7.2	3.4–57.2

potentially useful parameter since it follows exactly the same pattern every year, while local climate, i.e., the typical temperatures and precipitation regime of a region may be strongly affected by major global disturbances (such as El Niño and the Southern oscillation, Diaz & Markgraf, 2000). Also, the availability of food may be critical for controlling the energetically costly reproductive processes (Aldridge, 1983).

The current results show that water temperatures within the April range (early autumn) of a temperate zone have an inhibitory influence on the frequencies of both copulation and spawning in *Pomacea canaliculata*. This inhibition is not affected by changes in photoperiod length, and it is direct in the sense that it is not mediated through a decrease in the availability of food. However, food re-

striction was also able to induce a significant decrease in the frequencies of copulation and spawning in *P. canaliculata* individuals maintained in conditions of warm water and long photoperiod. It is remarkable that the number and size of eggs in each spawn was not altered by any treatment nor was the percentage of fertile eggs. It seems therefore that both fertilization and the oviductal formation of oocyte envelops (Catalán et al., 2002) are not impaired under the conditions of the current experiments, but that the number of oocytes produced is drastically reduced by both low temperature and food restriction. In the end, the energetically costly production of eggs was thus reduced to less than half.

Both the decrease of water temperature and availability of food may act either as proximate factors (with imme-
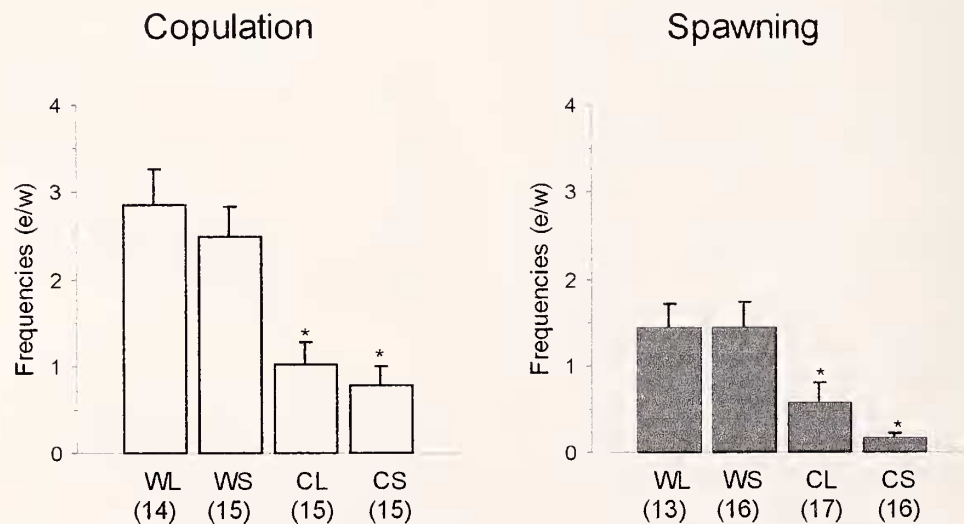
Copulation

Spawning

Figure 2. Effects of combinations of either warm (W) or cold (C) water, and of either long (L) or short (S) photoperiod on the mean frequencies (± SE) of copulation and spawning (episodes/week by mating pair). Number of cases is indicated between parentheses. Asterisks indicate (in the same panel) significant differences ($P < 0.05$, Kruskal-Wallis Test) from both warm water groups (WL and WS); other differences were not significant.

Table 2

Spawn measurements in females maintained under combinations of either warm (W) or cold (C) water, and of either long (L) or short (S) photoperiod. Results are expressed as the "weighted" mean (± SE) and the range of the mean values of individual females.

	WL (10 females, 36 spawns)		WS (15 females, 52 spawns)		CL (8 females, 18 spawns)		CS (5 females, 6 spawns)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Number of eggs per spawn	158.4 ± 21.8	51.0–282.0	193.4 ± 20.6	58.1–365.5	86.0 ± 25.9	31.0–229.0	77.8 ± 15.9	13.0–119.0
Mean egg volume (mm³)	9.6 ± 0.4	7.9–11.6	9.2 ± 0.4	6.2–11.8	11.9 ± 1.7	7.2–21.5	12.2 ± 1.1	8.9–15.7
Spawn volume (mm³)	1613.8 ± 236.3	523.7–3016.9	1835.1 ± 188.6	616.9–2994.8	1303.5 ± 447.4	94.2–4106.5	1086.9 ± 295.9	204.8–2199.9
% of developing embryos	48.4 ± 7.2	0.0–73.6	51.0 ± 5.6	1.0–89.1	36.2 ± 7.9	0.0–72.3	39.1 ± 10.7	0.0–67.6

diate beneficial or detrimental effects) or as ultimate factors (with effects on evolution of the reproductive strategy) (Baker, 1938). We suggest that these environmental factors are acting proximately in *P. canaliculata* since this and other *Pomacea* species reproduce throughout the year in the tropical and subtropical zones of Brazil and Paraguay (Hylton Scott, 1957; Fausto Filho, 1962, 1965; Milward de Andrade et al., 1978), and since *P. canaliculata* may shift from seasonal reproduction and iteroparity to both continuous reproduction and semelparity, provided that summer water temperatures are artificially maintained (Estebenet & Cazzaniga, 1992).

It has been proposed that day length may be of lesser importance than the preceding factors to cue the termination of reproductive processes in aquatic animals (Daly & Wilson, 1983). However, one can speculate that days that are becoming shorter may be more significant than days becoming longer in cueing reproductive processes in *Pomacea canaliculata* since the individuals are buried in the mud (and thus unable to perceive light) at the time when days become longer. Nevertheless, our results indicate that a short day length does not provoke by itself any effect on the frequencies of both copulation and spawning in non-buried animals, nor does it potentiate the effects of low temperatures on these frequencies.

In general, the relationships between these seasonally changing parameters with spawning and with other aspects of biological fitness (e.g., survival, fecundity, and growth) have received considerably more attention (Estebenet & Martín, 2002) than their relation to the seasonal changes in copulatory activity, probably because such behavior is often difficult to evaluate in nature (Aldridge, 1983). In the laboratory, however, we have observed (Albrecht et al., 1996, and this paper) that copulation occurs two to three times a week in reproductively active *P. canaliculata* and a typical copulatory episode lasts 10–12 hours, during which the male stops feeding. Then, the magnitude of such "negative" reproductive investment on the part of the male should also be kept in mind when adaptation to winter conditions is considered.

We conclude that both food restriction and low water temperatures are responsible for the seasonal arrest of copulatory and spawning behaviors in *Pomacea canaliculata*. However, the decrease in water temperature that occurs in autumn seems to be the critical factor that causes this seasonal arrest, since food availability is unlikely to decrease without a previous or simultaneous decrease in water temperature. Shortening of the photoperiod may be of little or no importance in determining the seasonal arrest of reproductive activities in this species.

of Mendoza, and from the National Research Council of Argentina, of which ACV is an established member.

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